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ORIGINAL ARTICLE

Irreversible electroporation of the pancreas in swine: a pilot study

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Abstract

Background: Irreversible electroporation (IRE) is a novel, non-thermal method of tissue ablation using short pulses of high-voltage DC current to ablate tissue.

Methods: Irreversible electroporation of the pancreas was performed in four domestic female swine using two monopolar probes spaced 9–15 mm apart. Ninety pulses of 1500 V/cm were delivered for each ablation.

Results: All animals survived for their designated times of 2 h ($n = 1$), 2 days ($n = 1$) and 14 days ($n = 2$), respectively. No procedure-related complications occurred. Three animals in which probes had been spaced at intervals of 10 ± 1 mm showed evidence of irreversible ablation, with ablation height ranging from <10 mm to 21 mm and ablation width ranging from <10 mm to 16 mm by gross appearance and triphenyltetrazolium chloride (TTC) staining. The only animal in which probes had been spaced at intervals of 15 mm did not show evidence of irreversible ablation at 2 weeks. This may be secondary to the wider probe spacing and relatively low voltage, which results in a mostly reversible form of electroporation without cell death.

Conclusions: Irreversible electroporation appears to be a safe method for pancreas tissue ablation. Staining with TTC can predict the zone of IRE ablation within 2 h of treatment.

Keywords

pancreas, ablation, irreversible, electroporation

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Introduction

Irreversible electroporation (IRE) is a novel ablation technology that utilizes short pulses of high-voltage electrical energy for tissue ablation. It is performed by placing electrodes into the tissue and delivering 90 pulses of 1000–3000 V/cm DC energy between the electrodes. Cell death is initiated via the creation of micropores in the cell membrane.[1]

Irreversible electroporation is a non-thermal form of ablation. Previous preclinical studies in swine liver demonstrated the ability of IRE to ablate tissue immediately adjacent to hepatic veins without evidence of heat sink. Hepatic arteries, portal veins and bile ducts within the ablation zone appear more resistant to the effects of IRE.[1–3]

The aim of the current study was to evaluate the feasibility and safety of performing IRE ablations *in vivo* in the pancreas in a swine model. Additionally, the ability of triphenyltetrazolium chloride (TTC) staining to predict the zone of IRE ablation was evaluated.

Materials and methods

This study was approved by our institution's Animal Care and Use Committee.

General anaesthesia was induced in four domestic female swine using glycopyrrolate 0.003 mg/kg i.m., telazol 5 mg/kg i.m. and xylazine 2 mg/kg i.m., and sodium thiopental 20 mg/kg i.v. as needed. Animals were intubated to facilitate mechanical ventilation. General anaesthesia was maintained with 2–4% isoflurane in oxygen. Pre-emptive analgesia was administered using buprenorphine 0.03 mg/kg i.m. The adequacy of anaesthesia was determined according to jaw tone and pedal reflex. Anaesthesia was increased if any response to stimuli was noted. A baseline evoked motor response was obtained with a nerve stimulator positioned

near the ulnar nerve. Animals were monitored intraoperatively using telemetry and pulse-oximetry.

The abdominal wall was aseptically prepared using betadine scrub and solution. Prior to incision, animals received a single dose of cephazolin 40 mg/kg i.v. A 12-cm incision was made in the midline of the abdomen and the peritoneal cavity entered. Pancuronium 0.1 mg/kg was administered to achieve reversible paralysis as monitored by evoked motor response.

The segment of pancreas abutting the superior mesenteric vein and duodenum was identified and two monopolar IRE electrodes were positioned within the pancreatic parenchyma, 10–15 mm apart. The active portion of the electrodes was exposed for a length of 20 mm. Electrode positioning was confirmed by intraoperative ultrasound. Pulses of 1500 V/cm were delivered between the electrodes in 100-microsecond (ms) pulses as determined by the manufacturer's treatment software (NanoKnife®; Angio Dynamics, Inc., Queensbury, NY, USA). Ninety pulses were delivered per ablation. The pulses were delivered in groups of 10 with a 250-ms pause between pulses.

Muscle relaxation was reversed using neostigmine 0.044–0.066 mg/kg i.v. and atropine 0.005 mg/kg i.v. Animals were recovered. Postoperative pain control was achieved using Fentanyl transdermal patches 75 mcg/h and tramadol 150 mg administered by mouth twice daily for 3 days and as needed.

Phlebotomy for amylase, lipase, AST (aspartate aminotransferase), ALT (alanine aminotransferase) and bilirubin was performed pre-procedure while the animals were under anaesthesia and on postoperative days 2 and 5 in surviving animals.

Once the target survival times of 2 h ($n = 1$), 2 days ($n = 1$) and 14 days ($n = 2$) had been reached, animals were anaesthetized as previously described and euthanized with pentobarbital 100 mg/kg. Laparotomy was performed and the pancreas was recovered. The area of ablation within the pancreas was sectioned at 5-mm intervals for gross evaluation. The 5-mm section in the centre of the ablation cavity was stained with TTC. The remaining sections were fixed in formalin. The height and width of ablation zones were measured. Histological analysis was performed following haematoxylin and eosin (H+E) staining.

Results

All four swine survived to the designated times and no complications were encountered. Treatment parameters and ablation sizes are shown in Table 1. In all animals, the electrodes were placed with the assistance of a 10-mm spacer. Although the electrodes all entered the pancreatic tissue at spacing of exactly 10 mm, the final locations of the active portion of the electrode tips varied slightly as documented by intraoperative ultrasound. The distance between the electrode tips ranged from 9 mm to 15 mm (Table 1).

Immediately following IRE, the ablated pancreatic tissue showed gross evidence of oedema and haemorrhage (Fig. 1).

At 2 h there was histological evidence of haemorrhagic necrosis. Staining with TTC showed a clear demarcation between viable tissue, which retained the TTC stain, and non-viable tissue, which did not retain the TTC stain.

At 48 h the zone of ablation remained well demarcated by gross evidence, H+E staining and TTC staining. The blood vessels and the pancreatic ducts appear to be more resistant to the effects of IRE (Fig. 2).

At 2 weeks, the animal in which the electrodes had been spaced 10 mm apart showed evidence of fibrosis within the ablation zone (Fig. 3). No histological changes were identified at 2 weeks in the animal in which the electrode tips had been spaced 15 mm apart.

Serum amylase and lipase were measured in the surviving animals at baseline (2380 [standard deviation, SD 620] and 5.8 [SD 5.7], respectively), at postoperative day 2 (2802 [SD 111] and 32 [SD 17], respectively) and at postoperative day 5 (2420 [SD 511] and 101 [SD 58], respectively). There was no clinical evidence of pancreatitis in any of the animals. All the animals exhibited minimal pain by demonstrating normal activity and normal feeding habits.

Discussion

Irreversible electroporation refers to the delivery of short pulses of high-voltage electrical energy to the target tissue, which results in micropore formation in the lipid bilayer of cells and subsequent cell death. Electroporation has also been used in a reversible manner to improve the delivery of bleomycin in the treatment of squamous cell carcinoma of the head and neck.[4–8] Rubinsky *et al.* demonstrated the ability of IRE to kill hepatocytes in swine liver with preservation of the portal structures. They also demonstrated hepatocyte necrosis immediately adjacent to large veins within the ablation zone, which were unaffected by the heat sink effect normally seen with thermal ablation techniques.[1]

Table 1 Irreversible electroporation ablations created with two monopolar probes and 90 pulses of 1500 V/cm *in vivo* in swine pancreas

Location	*n*	Exposure[a]	Distance[b]	Survival	Ablation zone[c]
Pancreas	1	20 mm	10 mm	2 h	15 × 16 mm
Pancreas	1	20 mm	9 mm	2 days	21 × 12 mm
Pancreas	1	20 mm	10 mm	2 weeks	<10 × <10 mm
Pancreas	1	20 mm	15 mm	2 weeks	Not visualized

[a]Exposure refers to the length of the electrode that is active during the delivery of treatment
[b]Distance between monopolar electrodes
[c]The width and height of ablation zones were measured following triphenyltetrazolium chloride staining and fixation

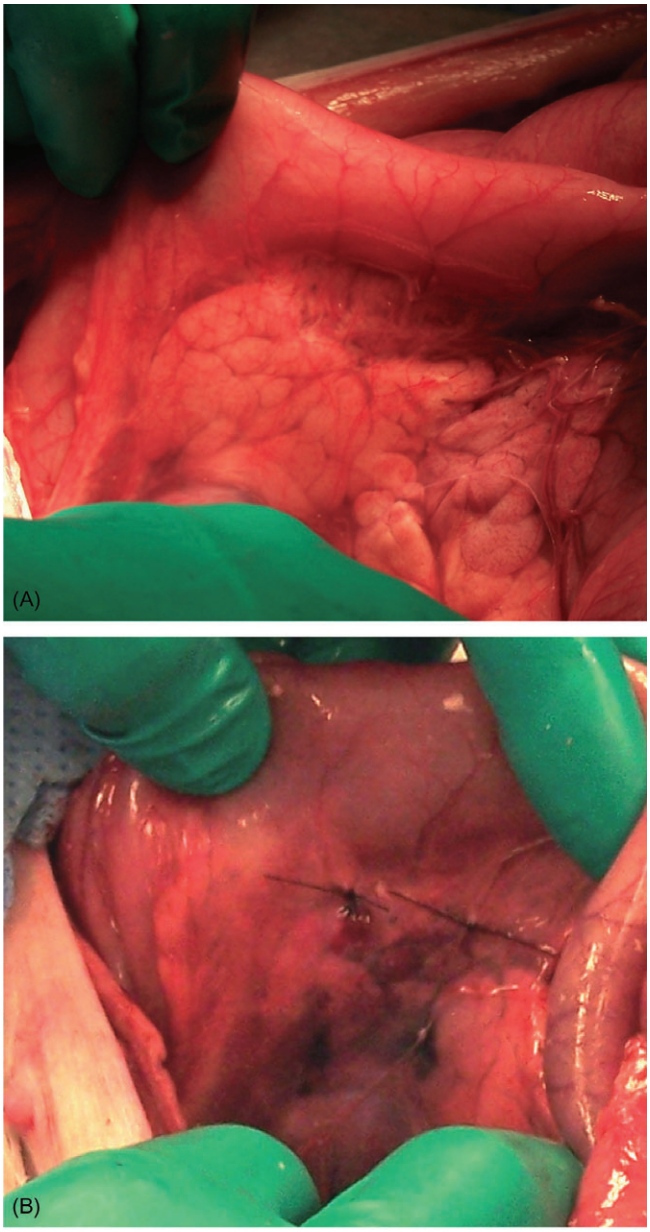

Figure 1 Gross intraoperative photographs of one swine pancreas (A) immediately before irreversible electroporation (IRE) showing normal pancreas, and (B) immediately after IRE, showing an area of oedema and haemorrhage corresponding to the area of ablation

Lee *et al.* corroborated the findings of Rubinsky's group and demonstrated the ability to deliver the IRE treatment percutaneously in swine liver.[2]

Miller *et al.* reported the ability of IRE to ablate human hepatocarcinoma cells *in vitro*. They showed that multiple pulses appeared to be more effective in cancer cell ablation than the delivery of equivalent energy in a single pulse.[9]

Al-Sekere *et al.* reported complete regression using IRE in 12 of 13 cutaneous tumours implanted in mice. Their work confirmed

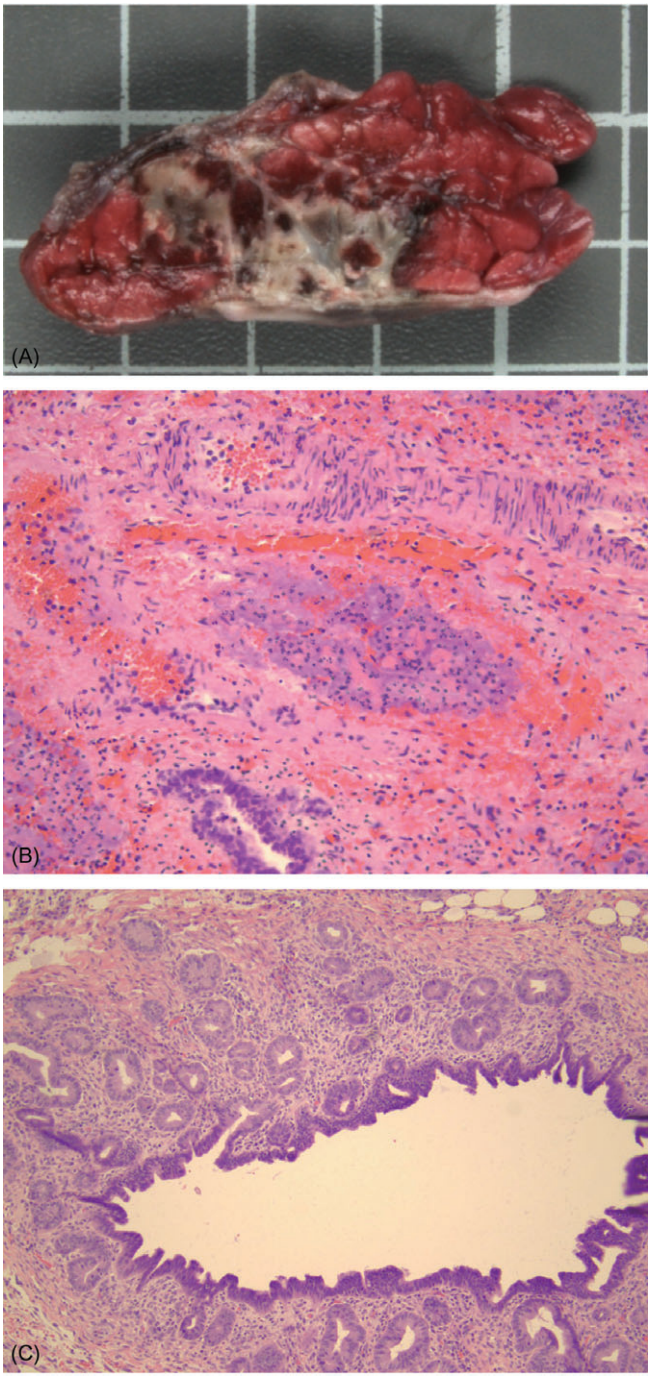

Figure 2 Pancreatic tissue at 2 days after irreversible electroporation (IRE). (A) Fixed tissue after triphenyltetrazolium chloride (TTC) staining. The red dye is retained by viable cells. Areas devoid of red dye represent regions of cell necrosis. (B) Pancreatic tissue at 20× original magnification after haematoxylin and eosin (H+E) staining. Pancreatic acinar tissue is destroyed with relative preservation of the blood vessels and pancreatic ducts. (C) Pancreatic tissue at 10× original magnification after H+E staining, demonstrating preservation of the pancreatic duct within the zone of IRE ablation

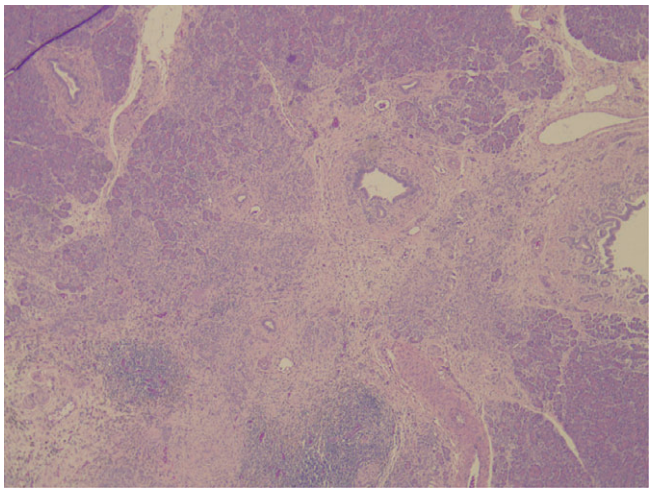

Figure 3 Pancreatic tissue at 2 weeks after irreversible electroporation shown at 4× original magnification reveals scarring in the ablation zone with preservation of the pancreatic ducts

improved results with cancer cell death using multiple short pulses of electrical energy.[10]

Our group has previously reported the safety and efficacy of performing IRE ablations in the liver hilum with preservation of the portal vein, bile duct and hepatic artery within the ablation zone. In addition, we reported the ability of TTC staining to accurately predict the zone of IRE ablation as early as 15 min following ablation in liver.[3]

Irreversible electroporation requires the insertion of electrodes into the target tissue for the delivery of the treatment. As with other forms of ablation (e.g. microwave and radiofrequency ablation), bleeding can occur at the electrode puncture site. The high-voltage current needed for ablation is unique to IRE. This necessitates reversible chemical paralysis to prevent muscle contraction. Although cardiac arrhythmias have been reported, the risk of arrhythmia can be minimized with cardiac synchronization. No IRE complications were encountered in our study.

The current paper reports a pilot study demonstrating the safety of IRE in swine pancreas. All of the animals survived to the designated time without clinical or laboratory evidence of pancreatitis. We believe the lack of pancreatitis can be explained in several ways. Firstly, the area of tissue ablated is relatively small and focal. Secondly, the pancreatic duct appears to be more resistant to the effects of IRE. Finally, the time-points selected for phlebotomy were not ideal for identification of mild pancreatitis. Future studies should include phlebotomy at earlier time-points in order to detect any subclinical pancreatitis.

Triphenyltetrazolium chloride staining is able to predict the zone of IRE ablation in the pancreas as early as 2 h after therapy.

Histological correlation confirmed cell death in pancreatic tissue following IRE in three of the four animals in our study. The fourth animal was the only subject in which the monopolar electrodes were more widely spaced, at 15 mm vs. 10 mm. We believe that the lack of histological changes at 2 weeks in this animal may be explained by technical issues relating to the relatively wide spacing of the electrodes and the use of a relatively low voltage (1500 V/cm), which resulted in a mostly reversible form of electroporation without cell death. Local tissue repair cannot be entirely excluded as a contributing cause of these findings.

In summary, IRE appears to be safe and effective for the ablation of pancreatic tissue. Staining by TTC is able to predict the zone of IRE ablation in the pancreas as early as 2 h following treatment. Further studies are needed to determine the optimal electrode spacing and voltage for creating IRE ablations in the pancreas. Additional studies will be needed to determine the efficacy of IRE for the ablation of pancreatic tumours.

Acknowledgements

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Conflict of interest

None declared.

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